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# Interactive Effects of Temperature, pH, and Water Activity on the Growth Kinetics of Shiga Toxin–Producing *Escherichia coli* O104:H4†

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## ABSTRACT

The risk of non-O157 Shiga toxin–producing *Escherichia coli* strains has become a growing public health concern. Several studies characterized the behavior of *E. coli* O157:H7; however, no reports on the influence of multiple factors on *E. coli* O104:H4 are available. This study examined the effects and interactions of temperature (7 to 46°C), pH (4.5 to 8.5), and water activity ($a_w$; 0.95 to 0.99) on the growth kinetics of *E. coli* O104:H4 and developed predictive models to estimate its growth potential in foods. Growth kinetics studies for each of the 23 variable combinations from a central composite design were performed. Growth data were used to obtain the lag phase duration (LPD), exponential growth rate, generation time, and maximum population density (MPD). These growth parameters as a function of temperature, pH, and $a_w$ as controlling factors were analyzed to generate second-order response surface models. The results indicate that the observed MPD was dependent on the pH, $a_w$, and temperature of the growth medium. Increasing temperature resulted in a concomitant decrease in LPD. Regression analysis suggests that temperature, pH, and $a_w$ significantly affect the LPD, exponential growth rate, generation time, and MPD of *E. coli* O104:H4. A comparison between the observed values and those of *E. coli* O157:H7 predictions obtained by using the U.S. Department of Agriculture Pathogen Modeling Program indicated that *E. coli* O104:H4 grows faster than *E. coli* O157:H7. The developed models were validated with alfalfa and broccoli sprouts. These models will provide risk assessors and food safety managers a rapid means of estimating the likelihood that the pathogen, if present, would grow in response to the interaction of the three variables assessed.

Illnesses caused by Shiga toxin–producing *Escherichia coli* (STEC), both O157 and non-O157 strains, continue to be a significant concern to the food industry, regulatory agencies, and the public. This pathogen can cause both sporadic cases and outbreaks of diarrhea with abdominal cramping that may lead to potentially life-threatening hemorrhagic colitis as well as hemolytic-uremic syndrome, especially in persons who have a weak immune system. *E. coli* O157:H7 is the most common predominant serotype associated with the most severe form of the disease *(26)*. However, illnesses and disease outbreaks linked to non-O157 STEC strains outnumber O157 STEC cases in the United States *(6, 7, 12)*. In a report from the Centers for Disease Control and Prevention, Scallan et al. *(25)* estimated that 175,000 illnesses are caused by foodborne STEC infections per year, with 64% of these infections linked to non-O157 STEC. A large number of non-O157 STEC–associated illnesses and disease outbreaks have been reported in several countries, including the United States, Australia, Japan, and Europe *(22)*. In Germany, a large outbreak due to STEC O104:H4 implicating sprouts as the vehicle of transmission that resulted in 3,842 cases of illness was reported in 2011 *(4)*. Thus, there has been a growing public health concern about the risk of non-O157 STEC strains.

Ruminants can be colonized and are recognized as asymptomatic carriers of both O157 and non-O157 STEC, with cattle being the natural, principal reservoir *(5, 23)*. Counts of STEC as high as 8 log CFU/g have been found in the excreta of colonized cattle *(3, 17)*. The likelihood of infections in humans with non-O157 STEC is significantly higher than that of infections with O157 STEC because of the high prevalence of the former in animals and as contaminants in foods *(27, 28)*. Infections may occur in humans when they come in contact with infected animals or consume inadequately cooked beef, vegetables, and dairy products contaminated by the feces of infected cattle. Many studies have reported the prevalence, detection, and characterization of non-O157 STEC *(1, 2, 6, 13–16, 24)*.

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Previous studies have investigated the effects of initial pH, sodium chloride (water activity [$a_w$]), sodium nitrite, and phosphates on the growth of enteropathogenic *E. coli* *(20, 21)*. Buchanan and Klawitter *(11)* examined the effects and interactions of temperature, pH, and sodium chloride on the growth kinetics of *E. coli* O157:H7 and reported that the three variables interacted to affect primarily the lag phase duration (LPD) and generation time (GT). While several studies *(8–10)* also characterized the behavior of *E. coli* O157:H7, there appear to be no reports on the influence of multiple factors on *E. coli* O104:H4. Accordingly, the objectives of the present work were to quantitatively assess the effects and interactions of temperature, pH, and $a_w$ on the growth kinetics of *E. coli* O104:H4 in a model system and to estimate its growth potential in foods. The goal was to develop a model that could be used to predict the growth of this pathogen within the boundaries of the variables.

## MATERIALS AND METHODS

**Bacterial strains and culture preparation.** The cocktail of three *E. coli* O104:H4 strains, obtained from the Centers for Disease Control and Prevention, used in the study comprised 2009EL-2050, 2009EL-2071 (both clinical isolates, Republic of Georgia, 2009), and 2011C-3493 (clinical isolate, Germany outbreak, 2011). Frozen stock cultures were maintained at −80°C in brain heart infusion broth (BHI; BD, Sparks, MD) supplemented with 15% (vol/vol) glycerol (Sigma-Aldrich Co., St. Louis, MO). Prior to initiating the study, each thawed culture (100 μl) was transferred into 10 ml of BHI broth in 50-ml tubes and incubated for 24 h at 37°C. These working cultures were maintained in BHI at 4°C and transferred every 2 weeks.

One day before the experiment, each culture (100 μl) was inoculated into 50 ml of BHI broth in 250-ml Erlenmeyer flasks and incubated for 18 to 20 h at 37°C on a rotary shaker (150 rpm) to obtain stationary-phase cells. The bacterial population in each cell culture was determined by manually plating appropriate dilutions in 0.1% (wt/vol) peptone water (PW; BD), in duplicate, onto tryptic soy agar (TSA; Teknova, Hollister, CA) plates. One milliliter of each culture was transferred into 7 ml of PW to yield an equal mixture of strains at a level of ca. 9 log CFU/ml. This cocktail was diluted to about 5 log CFU/ml in PW prior to inoculation of the test flasks.

**Experimental design.** A central composite design was utilized to assess the effects and interactions of incubation temperature (7 to 46°C), initial pH (4.5 to 8.5), and $a_w$ (0.95 to 0.99). The range of the initial pH or $a_w$ was chosen as representing culture conditions under which, when tested individually, the pathogen would grow. Growth kinetics studies for each of the 23 variable combinations (Table 1) were performed in duplicate after selecting at random, and two replications were conducted. The growth data were used to develop predictive models that describe the effects and interactions of these parameters on LPD (in hours), exponential growth rates (EGR, in log CFU per hour), GT (in hours), and maximum population densities (MPD, in log CFU per milliliter) of *E. coli* O104:H4.

**Experimental procedure.** Prior to sterilization, the selected $a_w$ and pH of BHI broth (Table 1) were adjusted by the addition of NaCl and 10 N HCl or 50% KOH, respectively. The medium was dispensed in 50-ml portions into 250-ml Erlenmeyer flasks, capped with foam plugs, and sterilized by autoclaving for 15 min at 121°C. After sterilization, each flask was inoculated with 0.5 ml of the diluted cocktail to obtain initial population densities of approximately 3 log CFU/ml. Thereafter, flasks were incubated at the selected temperatures (Table 1) on a rotary shaker set at 150 × *g*. At intervals that were appropriate for the culture conditions, duplicate 1.0-ml samples were withdrawn (maximum sampling time, 96 h), diluted as needed in PW, and surface plated in duplicate on BHI agar. All plates were incubated at 37°C for 24 h before colonies were counted.

**Data processing and model development.** Viable cell counts were transformed to log values, and growth curves were generated by plotting cell counts versus incubation time. The growth data were fitted with the modified Gompertz model (equation 1) with a curve-fitting program (DataFit for Windows, version 7.1, Oakdale Engineering, Oakdale, PA).

$$L(t) = A + C_{exp}\{-\exp[-B(t-M)]\} \quad (1)$$

where $L(t)$ is the log count of bacterial population at time $t$ in log CFU/gram, $A$ is the asymptotic log count of bacterial population as time decreases indefinitely (i.e., initial level) in log CFU/gram, $C$ is the asymptotic amount of growth that occurs as time increases indefinitely (i.e., number of log cycles of growth) in log CFU/gram, $M$ is the time at which the absolute growth rate is maximum (in hours), and $B$ is the relative growth rate at M, in (log CFU/gram)/hour.

The Gompertz parameter values ($A$, $B$, $C$, and $M$) were used to calculate the growth kinetic parameters LPD, EGR, GT, and MPD, as described previously *(18, 19)*, as follows: (i) EGR (in log CFU/hour) = $BC/e$; (ii) GT (in hours) = log (2)$e/BC$; (iii) LPD (in hours) = M − (1/$B$); (iv) MPD (in log CFU/milliliter) = $A + C$.

The growth kinetic parameters were analyzed by using the generalized linear model as well as regression procedures of the Statistical Analysis System to generate second-order response surface models to describe the LPD, EGR, GT, and MPD of *E. coli* O104:H4 as a function of temperature, pH, and $a_w$ as controlling factors. Each quadratic response surface model was represented by a polynomial equation as follows:

$$\begin{aligned}\text{LPD, EGR, GT, or MPD} = {} & C1 + C2(\text{temp}) + C3(\text{pH}) \\ & + C4(a_w) + C5(\text{temp})(\text{pH}) \\ & + C6(\text{temp})(a_w) \\ & + C7(\text{pH})(a_w) + C8(\text{temp})^2 \\ & + C9(pH)^2 + C10(a_w)^2\end{aligned} \quad (2)$$

where $C_1$ through $C_{10}$ are estimated coefficients.

The polynomial equations were entered into a spreadsheet, and predictions of LPD, EGR, GT, and MPD of *E. coli* O104:H4 were obtained for the ranges of the matrices.

**Model validation.** Validation of the model ultimately depends on its ability to predict the behavior of the pathogen in foods and enables the researchers to test the effectiveness of the models. Therefore, the potential growth of *E. coli* O104:H4 in alfalfa and broccoli sprouts was evaluated. Alfalfa and broccoli sprouts were obtained from a local supplier. Portions (3 g) were weighed into filtered stomacher bags. The bags were packaged into foil packets and irradiated to a dose of 6 kGy at 19 ± 1°C to remove background flora. Irradiated samples were kept refrigerated at 8°C until use (approximately 1 week). After inoculation with approximately 3 log CFU/g of 0.1% PW–washed cultures, bags with sprouts were gently tumbled to ensure even distribution

TABLE 1. *Effect of growth conditions on observed and estimated growth kinetic parameters of* E. coli *O104:H4*[a]

| | | | | EGR (log CFU/h) | | LPD (h) | | GT (h) | | MPD (log CFU/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temp (°C) | pH | $a_w$ | *n* | Observed | Estimated | Observed | Estimated | Observed | Estimated | Observed | Estimated |
| 7 | 6.5 | 0.97 | 2 | NG | | | | | | | |
| 15 | 5.3 | 0.99 | 2 | 0.11 (0.01) | 0.53 | 9.04 (0.03) | 14.91 | 2.83 (0.33) | 2.98 | 12.84 (0.52) | 13.27 |
| 15 | 6.5 | 0.97 | 2 | 0.11 (0.01) | 0.33 | 8.06 (2.94) | 11.52 | 2.83 (0.16) | 3.02 | 12.92 (1.21) | 13.69 |
| 15 | 7.7 | 0.99 | 2 | 0.19 (0.02) | 0.43 | 8.24 (3.20) | 10.23 | 1.61 (0.18) | 2.48 | 10.81 (0.20) | 11.84 |
| 15 | 7.7 | 0.96 | 2 | NG | | | | | | | |
| 15 | 5.3 | 0.96 | 2 | NG | | | | | | | |
| 15 | 7.7 | 0.96 | 2 | NG | | | | | | | |
| 21 | 6.5 | 0.97 | 2 | 0.35 (0.10) | 0.36 | 9.54 (4.11) | 7.78 | 0.90 (0.25) | 1.67 | 10.52 (0.08) | 11.65 |
| 27 | 6.5 | 0.95 | 2 | NG | | | | | | | |
| 27 | 4.5 | 0.97 | 2 | NG | | | | | | | |
| 27 | 5.3 | 0.96 | 2 | 0.19 (0.01) | 0.35 | 17.93 (0.79) | 19.47 | 1.62 (0.08) | 2.49 | 8.88 (0.10) | 9.48 |
| 27 | 5.3 | 0.98 | 2 | 0.79 (0.01) | 0.84 | 7.11 (0.77) | 5.30 | 0.38 (0.01) | 1.15 | 9.53 (0.08) | 10.65 |
| 27 | 6.5 | 0.97 | 12 | 0.34 (0.02) | 0.53 | 3.12 (1.25) | 5.47 | 0.89 (0.06) | 0.94 | 10.40 (0.66) | 10.64 |
| 27 | 6.5 | 0.99 | 2 | 0.74 (0.02) | 0.74 | 2.95 (0.03) | 4.70 | 0.41 (0.01) | 0.71 | 9.92 (0.04) | 10.42 |
| 27 | 7.7 | 0.96 | 2 | 0.28 (0.03) | 0.60 | 23.34 (3.47) | 26.54 | 1.07 (0.09) | 1.30 | 9.93 (0.08) | 10.14 |
| 27 | 7.7 | 0.98 | 2 | 0.63 (0.07) | 1.03 | 3.12 (0.43) | 5.41 | 0.48 (0.05) | 0.43 | 9.93 (0.12) | 10.47 |
| 27 | 8.5 | 0.97 | 2 | 1.22 (0.85) | 1.40 | 16.90 (2.65) | 21.18 | 0.32 (0.22) | 0.70 | 9.78 (0.14) | 10.31 |
| 32 | 6.5 | 0.97 | 2 | 0.71 (0.28) | 0.70 | 1.81 (2.47) | 4.04 | 0.45 (0.18) | 0.72 | 9.75 (0.32) | 10.43 |
| 38 | 5.3 | 0.96 | 2 | 0.13 (0.03) | 0.51 | 9.44 (3.68) | 14.77 | 2.47 (0.64) | 2.32 | 8.21 (0.14) | 9.01 |
| 38 | 5.3 | 0.99 | 2 | 0.85 (0.03) | 1.23 | 3.12 (0.14) | 7.09 | 0.35 (0.01) | 0.91 | 9.29 (0.03) | 10.33 |
| 38 | 6.5 | 0.97 | 2 | 0.94 (0.00) | 0.99 | 2.74 (0.15) | 3.17 | 0.32 (0.00) | 0.96 | 11.49 (0.26) | 11.01 |
| 38 | 7.7 | 0.96 | 2 | 0.85 (0.55) | 1.06 | 22.89 (3.30) | 23.73 | 0.45 (0.29) | 1.24 | 9.00 (0.30) | 10.59 |
| 38 | 7.7 | 0.99 | 2 | 1.35 (0.00) | 1.71 | 1.85 (0.66) | 6.08 | 0.22 (0.00) | 0.61 | 9.87 (0.20) | 10.76 |
| 46 | 6.5 | 0.97 | 2 | NG | | | | | | | |

[a] Observed values represent means (standard deviations) of 17 variable growth conditions, each performed in duplicate after selecting at random and two replications were conducted. Estimated values represent the upper limits of the mean confidence intervals (95%) of estimated growth kinetic parameters. EGR, exponential growth rates; LPD, lag-phase duration; GT, generation time; MPD, maximum population densities; NG, no growth (counts were at or below the initial inoculation levels at two consecutive sampling times).

of the inoculum in sprouts. Thereafter, the bags were stored at 4°C for 1 h to allow for pathogen attachment on sprouts. Bags were incubated at 15, 27, and 38°C, and samples were removed at predetermined time intervals for microbiological analysis. The performance of the models was measured by comparing the observed LPD, EGR, GT, and MPD values in sprouts with those predicted by the models.

## RESULTS AND DISCUSSION

The experimentally observed values for *E. coli* O104:H4 LPD, EGR, GT, and MPD as well as estimated LPD, GT, and MPD for a number of variable condition combinations under which growth occurred are given in Table 1. The observed MPD, in general, was dependent on the pH, $a_w$, and temperature of the growth medium. The cultures that exhibited <0.5-log-cycle increases, as well as those cultures in which population densities decreased from initial inoculation levels of about 3 log CFU/ml to an eventual nondetectable level for at least two consecutive sampling times, were designated as no growth (NG) cultures. *E. coli* O104:H4 did not grow or the bacterial population decreased gradually to nondetectable levels ($2.0 \times 10^1$ CFU/ml) in growth medium at pH 6.5 and $a_w$ of 0.97, incubated at 7 or 46°C. At 15°C, growth at $a_w$ of 0.96 was restricted regardless of the pH (5.3 or 7.7) of the growth medium. At 27°C, cultures at pH 4.5 and $a_w$ of 0.97 controlled growth. Increasing pH of the growth medium stored at 27°C to 6.5 and decreasing $a_w$ to 0.95 also restricted growth. These observations suggest that pH interacted with $a_w$ to control growth or decrease population levels of *E. coli* O104:H4 to nondetectable levels. When the growth of the pathogen occurred, the population densities attained were >9 log CFU/ml. The levels were <9 log CFU/ml only when both pH and $a_w$ were considerably low, sufficiently low to restrict growth. For example, when the growth medium at pH 5.3 and $a_w$ of 0.96 was incubated at 27°C, the mean MPD attained was ca. 8.8 log CFU/ml. This was also true for *E. coli* O104:H4 incubated at 38°C. The most rapid growth occurred at 38°C, pH 7.7, and $a_w$ of 0.99, at which the observed LPD was 1.85 h and GT was 0.22 h. Under these conditions, the initial inoculation levels of 3 log CFU/ml attained population levels of ca. 10 log CFU/ml after 24 h. Growth was also rapid at 32°C in media at pH 6.5 and $a_w$ of 0.97, at which the LPD and GT were 1.81 and 0.45 h, respectively. In contrast, the slowest growth occurred at 27°C in the growth medium at pH 7.7 and $a_w$ of 0.96, under which conditions the time to reach ca. 10 log CFU/ml was 54 h and the LPD was 23.34 h.

Increasing temperature resulted in a concomitant decrease in LPD times. At pH 6.5 and $a_w$ of 0.97, the observed LPD times were 8.06, 3.12, and 2.74 h at 15, 27, and 38°C, respectively. Likewise, a parallel decrease in GT was observed as temperature increased; the values were

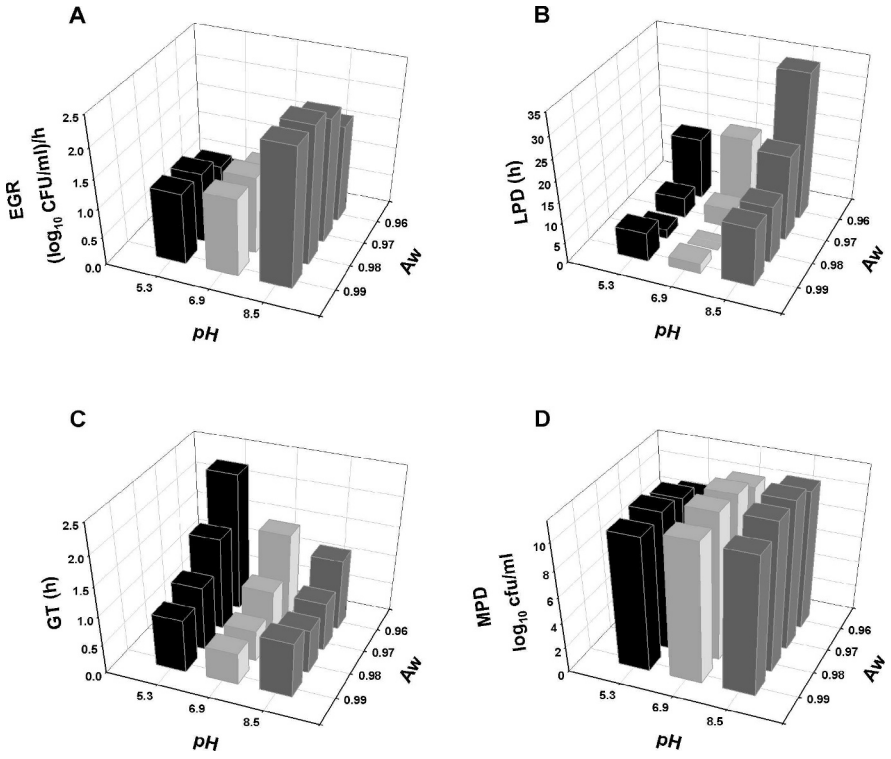


FIGURE 1. *Effects and interactions of pH and water activity combinations on LPD, EGR, GT, and MPD of* E. coli *O104:H4 in BHI broth at 38°C.*

2.83, 0.89, and 0.32 h at 15, 27, and 38°C, respectively. These data suggest the importance of decreasing temperature to delay LPD and increase GT. Higher pH of the growth medium tended to decrease the observed LPD and GT, and this effect was slightly more obvious at higher temperatures (Table 1). At 15°C and $a_w$ of 0.99, increasing the pH of the growth medium from 5.3 to 7.7 decreased LPD and GT values from 9.04 and 2.83 h to 8.24 and 1.61 h, respectively, while the decreases in values for LPD and GT at 38°C were 3.12 and 0.35 h to 1.85 and 0.22 h, respectively. Figure 1 depicts the relation among variables, i.e., the effect of pH and $a_w$ on the estimated LPD and GT of *E. coli* O104:H4 at 38°C. It was found that pH and $a_w$ have a combined (or interacted) effect on the LPD and the GT as well. The $a_w$ had substantial influence on the effect of pH on the estimated LPD and GT. At 38°C and pH 5.3, increasing $a_w$ from 0.96 to 0.99 decreased the estimated LPD and GT from 14.77 and 2.32 h to 7.09 and 0.91 h, respectively (Table 1). Decreases in LPD and GT were also observed at pH 8.5 of the growth medium at 38°C, at which estimated LPD and GT decreased from 34.64 and 1.21 h to 14.38 and 0.94 h, respectively, as $a_w$ increased from 0.96 to 0.99. A similar trend was observed at lower temperatures, when the increase of pH resulted in a parallel increase in estimated LPD and GT and the increase of $a_w$ resulted in a parallel decrease in estimated LPD and GT.

Due to the lack of published scientific literature, direct comparison of the growth kinetic values of *E. coli* O104:H4 obtained in the present study cannot be done. Published studies on the growth characteristics of *E. coli* O157:H7 with temperature, pH, sodium chloride, sodium nitrite, and/or humectant identity as controlling factors are available *(8–11)*. Increasing levels of sodium chloride resulted in parallel changes in LPD and GT only when several variables were limiting and the pH of the growth medium exhibited little impact on growth unless the pH was <5.5 *(11)*. In another study, addition of sodium nitrite exhibited significant inhibition at pH values of <5.5, and this inhibition was enhanced at lower temperatures *(10)*.

No-growth data were not included in the data for developing the response surface models for describing the LPD, EGR, GT, and MPD of *E. coli* O104:H4 as a function of temperature, pH, and $a_w$. Accordingly, growth kinetics data from 34 curves representing 17 variable combinations were used in developing the polynomial response models. The following polynomial models describe the effects of temperature, pH, and $a_w$ and their interaction on the growth kinetic parameters of *E. coli* O104:H4:

$$\begin{aligned}\text{EGR} = &-1{,}075.5(428.36) - 0.557(0.45)\times\text{temp}\\ &- 0.945(3.35)\times\text{pH} + 2{,}211.81(878.02)\times a_w\\ &+ 0.0106(0.01)\times\text{temp}\times\text{pH}\\ &+ 0.5307(0.45)\times\text{temp}\times a_w\\ &- 0.8548(3.23)\times\text{pH}\times a_w\\ &+ 0.00011(0.00)\times\text{temp}\times\text{temp}\\ &+ 0.1223(0.04)\times\text{pH}\times\text{pH}\\ &- 1{,}130.75(449.40)\times a_w\times a_w\end{aligned} \quad (3)$$

(Standard errors of estimated parameters are given in parentheses; $P < 0.0001$, $R^2 = 0.742$, adjusted $R^2 = 0.832$, mean standard error [MSE] = 0.0505, $F = 10.86$.)

TABLE 2. F *values for independent variables and their cross products for the quadratic models based on Gompertz growth kinetic parameters of* E. coli *O104:H4*[a]

| | EGR (log CFU/h) | | LPD (h) | | GT (h) | | MPD (log CFU/ml) | |
|---|---|---|---|---|---|---|---|---|
| Culture variable | *F* | Pr > *F* | *F* | Pr > *F* | *F* | Pr > *F* | *F* | Pr > *F* |
| Temp | 39.07 | <0.0001 | 0.63 | 0.4315 | 61.06 | <0.0001 | 54.58 | <0.0001 |
| pH | 18.88 | 0.0001 | 18.60 | 0.0001 | 30.06 | <0.0001 | 0.00 | 0.9458 |
| $a_w$ | 20.63 | <0.0001 | 72.31 | <0.0001 | 11.81 | 0.0016 | 3.02 | 0.0911 |
| temp × pH | 3.85 | 0.0580 | 4.17 | 0.0490 | 0.02 | 0.8881 | 10.06 | 0.0032 |
| temp × $a_w$ | 3.05 | 0.0900 | 13.59 | 0.0008 | 13.80 | 0.0007 | 0.23 | 0.6352 |
| pH × $a_w$ | 0.83 | 0.3698 | 20.55 | <0.0001 | 4.65 | 0.0383 | 1.63 | 0.2108 |
| temp × temp | 0.27 | 0.6100 | 10.90 | 0.0023 | 43.11 | <0.0001 | 15.41 | 0.0004 |
| pH × pH | 4.84 | 0.0348 | 73.89 | <0.0001 | 2.82 | 0.1025 | 13.60 | 0.0008 |
| $a_w$ × $a_w$ | 6.33 | 0.0168 | 48.55 | <0.0001 | 3.48 | 0.0706 | 8.96 | 0.0051 |

[a] EGR, exponential growth rates; LPD, lag-phase duration; GT, generation time; MPD, maximum population density; Pr > *F*, probability of a larger value of *F*; $a_w$, water activity.

$$\begin{aligned}\text{LPD} = {} & +\ 34{,}303.8(4{,}966.76) - 3.899(5.27) \times \text{temp} \\ & +\ 100.70(38.91) \times \text{pH} - 70{,}452.8(10{,}180.48) \times a_w \\ & +\ 0.0661(0.06) \times \text{temp} \times \text{pH} \\ & +\ 0.0168(5.22) \times \text{temp} \times a_w \\ & -\ 138.82(37.51) \times \text{pH} \times a_w \\ & +\ 0.0013(0.01) \times \text{temp} \times \text{temp} \\ & +\ 2.5995(0.48) \times \text{pH} \times \text{pH} \\ & +\ 36{,}305.9(5{,}210.80) \times a_w \times a_w \end{aligned} \quad (4)$$

(Standard errors of estimated parameters are given in parentheses; $P < 0.0001$, $R^2 = 0.886$, adjusted $R^2 = 0.948$, MSE = 6.791, $F = 29.24$.)

$$\begin{aligned}\text{GT} = {} & +\ 1{,}409.6(697.12) - 0.329(0.74) \times \text{temp} \\ & -\ 11.623(5.46) \times \text{pH} - 2{,}764.31(1{,}428.91) \times a_w \\ & +\ 0.00354(0.01) \times \text{temp} \times \text{pH} \\ & -\ 0.1001(0.73) \times \text{temp} \times a_w \\ & +\ 10.706(5.26) \times \text{pH} \times a_w \\ & +\ 0.00604(0.00) \times \text{temp} \times \text{temp} \\ & +\ 0.0588(0.07) \times \text{pH} \times \text{pH} \\ & +\ 1{,}365.31(731.38) \times a_w \times a_w \end{aligned} \quad (5)$$

(Standard errors of estimated parameters are given in parentheses; $P < 0.0001$, $R^2 = 0.834$, adjusted $R^2 = 0.969$, MSE = 0.1337, $F = 18.98$.)

$$\begin{aligned}\text{MPD} = {} & -\ 3{,}739.3(1{,}228.42) - 1.794(1.30) \times \text{temp} \\ & +\ 18.367(9.62) \times \text{pH} + 7{,}611.4(2{,}517.92) \times a_w \\ & +\ 0.0336(0.01) \times \text{temp} \times \text{pH} \\ & +\ 0.9853(1.29) \times \text{temp} \times a_w \\ & -\ 16.04(9.28) \times \text{pH} \times a_w \\ & +\ 0.00966(0.00) \times \text{temp} \times \text{temp} \\ & -\ 0.2757(0.12) \times \text{pH} \times \text{pH} \\ & -\ 3{,}858.3(1{,}288.78) \times a_w \times a_w \end{aligned} \quad (6)$$

(Standard errors of estimated parameters are given in parentheses; $P < 0.0001$, $R^2 = 0.760$, adjusted $R^2 = 0.878$, MSE = 0.415, $F = 11.94$.)

The *F* values obtained for the models were evaluated to assess the relative role of the effects and interactions of the three variables and are shown in Table 2. Several variables are highly significant ($P < 0.001$). The $R^2$, MSE, and *F* values for LPD, EGR, GT, and MPD suggest that the regression models satisfactorily describe the experimental data and would provide acceptable predictions of the growth kinetics values of *E. coli* O104:H4 at any combinations of the parameters within the temperature, pH, and $a_w$ assessed in this study.

Comparisons between the observed growth kinetics of *E. coli* O104:H4 with the predicted values for *E. coli* O157:H7 obtained with the USDA Pathogen Modeling Program (PMP) are shown in Table 3. The observed LPDs were 10 to 55 h shorter than those predicted by the PMP. Likewise, the observed GTs were shorter (0.32 to 2.83 h) than the PMP-predicted GTs (0.7 to 5.20 h). While the present model and the one available in the PMP were developed from data on bacterial growth obtained in broth and $a_w$ was adjusted with sodium chloride, it must be noted that the PMP model was developed in assays using *E. coli* O157:H7. Thus, serotype differences may have played a role in the short growth kinetic values observed in the

TABLE 3. *Comparison of observed growth kinetics of* E. coli *O104:H4 in broth compared with those predicted by the Agricultural Research Service (ARS) Pathogen Modeling Program for* E. coli *O157:H7*[a]

| | | | | LPD (h) | | GT (h) | |
|---|---|---|---|---|---|---|---|
| Temp (°C) | pH | $a_w$ | *n* | Observed | PMP | Observed | PMP |
| 15 | 6.5 | 0.97 | 2 | 8.06 (2.94) | 63.6 | 2.83 (0.16) | 5.2 |
| 27 | 6.5 | 0.97 | 12 | 3.12 (1.25) | 15.5 | 0.89 (0.06) | 1.2 |
| 38 | 6.5 | 0.97 | 2 | 2.74 (0.15) | 12.8 | 0.32 (0.00) | 0.7 |

[a] Observed values represent means (standard deviations) of observed growth kinetics. LPD, lag-phase duration; GT, generation time.

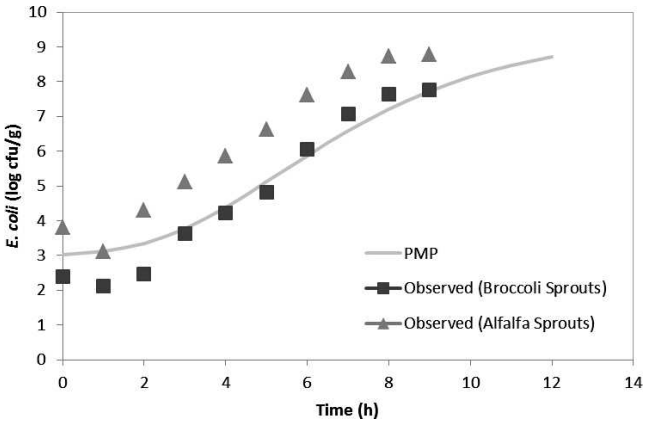


FIGURE 2. *Growth of* E. coli *O104:H4 at 38°C on sprouts and as predicted from the PMP at pH 6.5 and $a_w$ of 0.99.*

present study compared to the predictions from the PMP. The differences between the observed values and those predicted by PMP suggest that *E. coli* O104:H4 grows faster than *E. coli* O157:H7; therefore, the PMP model is not suitable for predicting the behavior of *E. coli* O104:H4.

**Evaluation of model performance.** The developed models were compared to growth of *E. coli* O104:H4 in alfalfa and broccoli sprouts at 38, 27, and 15°C. Growth of the pathogen at 38°C on sprouts and growth predicted from the PMP for *E. coli* O157:H7 at pH 6.5 and $a_w$ of 0.99 is depicted in Figure 2. Table 4 shows the experimentally observed growth kinetics values of EGR, LPD, GT, and MPD in sprouts. The pathogen grew readily in both alfalfa and broccoli sprouts at all temperatures, and MPDs of 7 to 9 log CFU/g were attained after 54, 24, and 9 h at 15, 27, and 38°C. As the incubation temperature increased from 15 to 38°C, a parallel decrease in LPD and GT was observed. The LPD and GT were used to calculate the expected time for the population density to increase 1,000-fold. This value ($T_{1,000}$) is the time required for the cell number to increase by 3 log (from 1 to 1,000 CFU/ml) and is considered to be a way to compare the influence of variables on LPD and GT as well as to compare observed and predicted growths. Table 5 shows a comparison of the $T_{1,000}$ observed in sprouts with values estimated by the growth model under similar conditions, pH 6.5 and $a_w$ of 0.99. The observed values for *E. coli* O104:H4 in sprouts at 27 and 38°C were found to compare favorably, implying reasonable estimation, with the corresponding values predicted by the model.

TABLE 5. *Comparison of growth of* E. coli *O104:H4 observed in sprouts with growth predicted by the model*

| | | $T_{1,000}$ (h)[a] | |
|---|---|---|---|
| Sprout | Temp (°C) | Observed | Predicted |
| Alfalfa | 38 | 5.03 | 7.92 |
| | 27 | 8.03 | 11.80 |
| | 15 | 45.19 | 33.80 |
| Broccoli | 38 | 5.02 | 7.92 |
| | 27 | 8.86 | 11.80 |
| | 15 | 38.57 | 33.80 |

[a] $T_{1,000}$, time for a 1,000-fold increase in pathogen numbers.

The predicted growth was slightly faster than the observed; therefore, the predictions of the model are located in the fail-safe side. At 15°C, slower growth was predicted in sprouts. Also, it is worth mentioning that the difference between observed and predicted $T_{1,000}$ values tended to be greater at 15°C than at 27 and 38°C. The low predicted growth kinetics values compared to the observed values at lower temperatures may be attributed to insufficient experimental data collected at low temperatures used in the model development. Additionally, comparatively more inherent variability of the physiological processes may occur at low temperatures. Nevertheless, the evaluation of the growth model suggests that the observed and predicted values compared well for growth at 27 and 38°C but not at 15°C. It appears that additional research efforts should be directed toward collection of additional growth data at lower temperatures to improve the model. The observation also suggests a need for additional studies to be conducted on additional food products to fully assess the performance of the models. It is worth pointing out that the cumulative effects of several additional extrinsic and intrinsic factors undoubtedly have an effect on the growth of the pathogen in foods and specific factors may play a significant role in a particular food. These basic concepts must be taken into consideration when refining a particular predictive model.

**Summary.** In the study reported herein, observed growth and no-growth culture condition combinations can be used with confidence to predict the behavior of the pathogen under different growth conditions. The response surface models developed can be used as a rapid means of estimating the effects and interactions of temperature, pH,

TABLE 4. *Gompertz growth kinetic values for* Escherichia coli *O104:H4 in sprouts*[a]

| Sprout | Temp (°C) | EGR (log CFU/h) | GT (h) | LPD (h) | MPD (log CFU/ml) |
|---|---|---|---|---|---|
| Alfalfa | 38 | 0.94 (0.15) | 0.33 (0.05) | 1.73 (0.92) | 9.55 (0.35) |
| | 27 | 0.59 (0.10) | 0.51 (0.09) | 2.93 (0.47) | 9.68 (0.20) |
| | 15 | 0.13 (0.01) | 2.30 (0.20) | 22.19 (0.54) | 9.08 (0.15) |
| Broccoli | 38 | 1.01 (0.20) | 0.30 (0.06) | 2.02 (0.55) | 9.02 (0.13) |
| | 27 | 0.53 (0.16) | 0.60 (0.18) | 2.86 (0.75) | 9.46 (0.48) |
| | 15 | 0.14 (0.01) | 2.09 (0.19) | 17.67 (0.76) | 9.63 (0.31) |

[a] Growth kinetic values are means (standard deviations). EGR, exponential growth rates; GT, generation time; LPD, lag-phase duration; MPD, maximum population density.

and $a_w$ on the growth of *E. coli* O104:H4. These models will provide risk assessors and food safety managers a rapid means of estimating the likelihood that the pathogen, if present, would grow in response to the interaction of the three variables assessed in this study. Also, the current models will play a significant role in product development to effectively design multiple food formulations that rely on intrinsic properties of foods to guard against *E. coli* O104:H4 as well as to assess the impact of altering any combination of food formulation variables. However, the models should not be used as a sole means of ensuring the microbiological safety of foods of interest. It is the processors' responsibility to use the predictions as guidelines and validate them in their products. Acquisition of data in foods to develop predictive models for specific food systems is an area for future research to increase the usefulness of the models by the food industry and regulatory agencies.

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